Journal of Experimental Botany, Vol. 70, No. 17 pp. 4429–4439, 2019
doi:10.1093/jxb/erz197 Advance Access Publication April 27, 2019

REVIEW PAPER

The function of S-nitrosothiols during abiotic stress in plants

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Received 3 December 2018; Editorial decision 15 April 2019; Accepted 22 April 2019

Editor: Renaud Brouquisse, Sophia Agrobiotech Institute, INRA, France

Abstract

Nitric oxide (NO) is an active redox molecule involved in the control of a wide range of functions integral to plant biology. For instance, NO is implicated in seed germination, floral development, senescence, stomatal closure, and plant responses to stress. NO usually mediates signaling events via interactions with different biomolecules, for example the modulation of protein functioning through post-translational modifications (NO-PTMs). S-nitrosation is a reversible redox NO-PTM that consists of the addition of NO to a specific thiol group of a cysteine residue, leading to formation of S-nitrosothiols (SNOs). SNOs are more stable than NO and therefore they can extend and spread the *in vivo* NO signaling. The development of robust and reliable detection methods has allowed the identification of hundreds of S-nitrosated proteins involved in a wide range of physiological and stress-related processes in plants. For example, SNOs have a physiological function in plant development, hormone metabolism, nutrient uptake, and photosynthesis, among many other processes. The role of S-nitrosation as a regulator of plant responses to salinity and drought stress through the modulation of specific protein targets has also been well established. However, there are many S-nitrosated proteins that have been identified under different abiotic stresses for which the specific roles have not yet been identified. In this review, we examine current knowledge of the specific role of SNOs in the signaling events that lead to plant responses to abiotic stress, with a particular focus on examples where their functions have been well characterized at the molecular level.

Keywords: Abiotic stress, antioxidant, nitric oxide, S-nitrosothiols, S-nitrosylation, signaling.

Introduction

Plants are continuously exposed to environmental changes that can alter the cellular redox homeostasis and hence potentially compromise plant survival and, in the case of crops, their final yield. These redox changes are a consequence of bursts of reactive oxygen and nitrogen species (ROS and RNS), chief among which are hydrogen peroxide (H_2O_2) and nitric oxide (NO), respectively. These reactive molecules have dual effects since at high concentrations they can induce nitro-oxidative stress with the potential capacity to induce cellular damage whilst at low concentrations they act as signaling molecules, leading to plant defense responses against the adverse conditions that the plant is facing(Begara-Morales *et al.*, 2016a). Consequently, in order to protect themselves, plants have to perceive these alterations in redox state and subsequently trigger the defense-signaling events. In this context, the metabolism of thiol groups has been proposed to be fundamental in redox

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responses to abiotic stress and during plant defense to pathogens (Foyer and Noctor, 2005; Spadaro *et al.*, 2010; Spoel and Loake, 2011; Zagorchev *et al.*, 2013). The oxidation of cysteine (Cys) residues appears to act as a redox sensor that perceives the environmental changes and triggers the redox signaling events that lead to plant defense (Spadaro *et al.*, 2010; Diaz-Vivancos *et al.*, 2015; Sevilla *et al.*, 2015; Begara-Morales *et al.*, 2016a). However, only a specific group of Cys residues that exhibit a low pKa are susceptible to oxidation and can therefore act as redox targets (Meng *et al.*, 2002; Diaz-Vivancos *et al.*, 2015; Sevilla *et al.*, 2015). These redox-sensitive Cys residues have different susceptibilities to several post-translational modifications (PTMs) mediated by RNS, including S-nitrosation (Hess *et al.*, 2005; Spadaro *et al.*, 2010; Begara-Morales *et al.*, 2016a; Umbreen *et al.*, 2018), which consists of the reversible addition of NO to a thiol group of a reactive Cys residue, leading to the formation of S-nitrosothiols (SNOs) (Hess *et al.*, 2005). In recent years, numerous pieces of evidence have shown that SNO formation is implicated in plant responses to abiotic stress via regulation of the functions of specific defense-related targets, especially within the antioxidant systems (Fancy *et al.*, 2017; Begara-Morales *et al.*, 2018). For example, the S-nitrosation of ascorbate peroxidase (APX) at Cys32 has been proposed to regulate plant responses to oxidative and salt stresses (Begara-Morales *et al.*, 2014a; Yang *et al.*, 2015), whilst S-nitrosation of S-nitrosoglutathione reductase 1 (GSNOR) at Cys10 has been proposed as a regulatory mechanism that mediates its degradation by selective autophagy, which as a consequence promotes seed germination and enhances plant tolerance to hypoxia stress (Zhan *et al.*, 2018).

With regards to the functioning of SNO, there is a general model of the signaling pathways triggered by SNOs during plant immune responses (Feechan *et al.*, 2005; Rustérucci *et al.*, 2007; Tada *et al.*, 2008; Wang *et al.*, 2009; Yun *et al.*, 2011; Cui *et al.*, 2018) but less information is available concerning the involvement of these signaling molecules during abiotic stress. In this review, we examine our current knowledge of the metabolism and functioning of SNOs during plant responses to adverse environmental conditions. We focus in particular on salinity and drought, as relatively more detailed molecular characterization of the functioning of SNOs has been determined for these two forms of stress.

Modulation of S-nitrosothiol levels

In order to understand the functioning of SNOs as molecular cues implicated in signaling events, it is first necessary to know the mechanisms involved in the regulation of their content.

Although S-nitrosation and S-nitrosylation are two terms commonly used to describe the formation of SNOs, S-nitrosation is more appropriate because it describes the formation—and transfer—of a nitroso group to a thiol to generate an SNO. S-nitrosylation is more related to the interaction of a nitrosyl group with metals (Smith and Marletta, 2012). In general terms, we can define SNOs as the result of the interaction of NO with the thiol group of a rare and reactive cysteine residue (Hess *et al.*, 2005). However, a direct interaction is not possible due to the low reactivity of the NO with non-radical molecules, and consequently the chemical mechanisms leading to SNO formation involve intermediate radicals (Broniowska and Hogg, 2012; Smith and Marletta, 2012; Lamotte *et al.*, 2014; Begara-Morales *et al.*, 2018). Hence, there are different reactions that can lead to SNO formation (Smith and Marletta, 2012). It is important to note that mass spectrometry has determined that a high number of S-nitrosated proteins are localized in different organelles such as peroxisomes, the nucleus, mitochondria, and the cytosol (Fares *et al.*, 2011; Chaki *et al.*, 2015; Hu *et al.*, 2015). Consequently, the reactions leading to SNO formation (Fig. 1) could take place at these subcellular locations. For instance, NO can interact with NO_2 to generate N_2O_3 (Kharitonov *et al.*, 1995; Keszler *et al.*, 2010), which is able to mediate the formation of protein-SNOs or low molecular weight SNOs (LMW-SNOs). Chief among the latter is the formation of S-nitrosoglutathione (GSNO) (Broniowska and Hogg, 2012; Smith and Marletta, 2012; Lamotte *et al.*, 2014; Begara-Morales *et al.*, 2018). In addition, SNOs are also able to mediate transnitrosylation reactions, by which they transfer the NO group to another thiol to generate a new SNO (Hess *et al.*, 2005; Broniowska and Hogg, 2012). A stress situation usually triggers the over-production of ROS and RNS, which in combination lead to a nitro-oxidative stress. In this situation, the peroxynitrite generated by the interaction of NO and $O_2^{\cdot-}$ can be broken down into NO_2 and $OH^{\cdot}$ radicals (Radi *et al.*, 2001; Szabó *et al.*, 2007, Broniowska and Hogg, 2012). These radicals can oxidize glutathione (GSH) and thiol groups in proteins to generate thyil radicals that in turn can interact with NO in a radical–radical interaction that also leads to formation of GSNO and SNOs (Hess *et al.*, 2005; Broniowska and

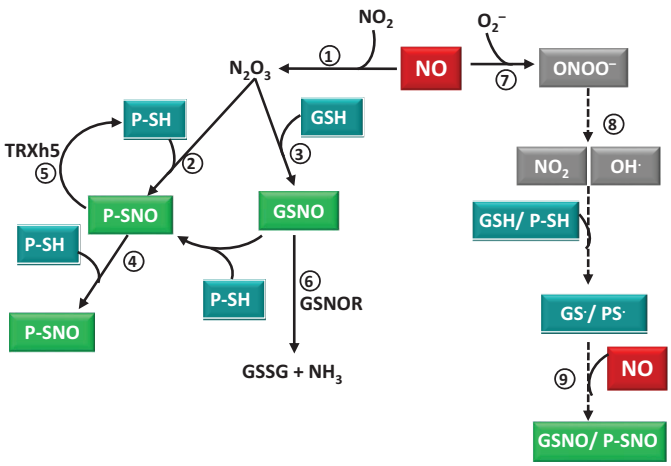

Fig. 1. Pathways of S-nitrosothiol formation. Nitric oxide (NO) can interact with NO_2 to generate N_2O_3 (1) that in turn is able to mediate the formation of protein S-nitrosothiols (P-SNO) (2) or S-nitrosoglutathione (GSNO) (3). P-SNOs can also generate new P-SNOs by transnitrosylation mechanisms (4). SNO content is controlled by specific denitrosylase enzymes such as thioredoxin h5 (TRXh5) (5) and indirectly by S-nitrosoglutathione reductase (GSNOR) (6). S-nitrosothiols can also be generated via formation of peroxynitrite ($ONOO^-$) (7) that can be broken down into intermediate radicals (8) that oxidize protein thiols and favor subsequent S-nitrosation by NO (9). GS, glutathionyl radical; GSH, glutathione; GSSG, oxidized glutathione; PS, thiolate group of the protein; P-SH, thiol group of the protein.

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Hogg, 2012; Smith and Marletta, 2012; Broniowska *et al.*, 2013; Lamotte *et al.*, 2014; Begara-Morales *et al.*, 2018) (Fig. 1).

Although SNOs can be broken down non-enzymatically (Holmes and Williams, 2000; Hogg, 2002), in order to act specifically as molecular cues involved in signaling events their content has to be tightly regulated. Consequently, the SNO signal is mainly controlled by specific enzymes, collectively named denitrosylases, that break down SNOs in both animals and plants (Benhar *et al.*, 2009; Anand and Stamler, 2012; Begara-Morales and Loake, 2016; Mata-Pérez and Spoel, 2019). In plants, the specificity and mode of action of these denitrosylases is just starting to be elucidated, with S-nitrosoglutathione reductase (GSNOR) and thioredoxin enzymes being key players in this process (Begara-Morales and Loake, 2016). GSNOR is an evolutionarily conserved enzyme from bacteria to mammals that is able to reduce GSNO to oxidized glutathione (GSSG) and NH_3 (Liu *et al.*, 2001; Feechan *et al.*, 2005) (Fig. 1). Consequently, GSNOR does not act by removing NO from a protein SNO, but it controls intracellular levels of GSNO and indirectly the level of total SNOs. This enzyme has been proposed to be involved in different cellular responses in animals (Liu *et al.*, 2004; Feechan *et al.*, 2005; Anand and Stamler, 2012; Beigi *et al.*, 2012; Cao *et al.*, 2015) and plants (Begara-Morales and Loake, 2016). In plants, a general strategy used to determine the importance of SNOs in signaling events has been the use of transgenic lines where GSNOR is either blocked or enhanced, leading to SNO accumulation or depletion, respectively. As a consequence of increased SNO levels, plants usually exhibit sensitivity to pathogen attack (Feechan *et al.*, 2005, Yu *et al.*, 2014) and an alteration of redox status that leads to an antioxidant response during abiotic stress responses (Kovacs *et al.*, 2016; Fancy *et al.*, 2017). In animal cells the thioredoxin/thioredoxin reductase (Trx/TrxR) system has been proposed to regulate NO homeostasis via the control of the extent of SNOs under different phatological and physiological conditions (Benhar *et al.*, 2008). The chemistry of denitrosylation by the Trx/TrxR system can be carried out by two potential reactions: via disulphide formation between the substrate and Trx, and via a transnitrosylation reaction in which the Trx is transiently S-nitrosated by the NO from the protein substrate (Benhar *et al.*, 2009; Begara-Morales *et al.*, 2016a; Mata-Pérez and Spoel, 2019). However, in plants there is much less information on the involvement of the thioredoxin enzymes in the control of the SNO-mediated signaling events (reviewed by Mata-Pérez and Spoel, 2019), with most of the available data being related to the function of thioredoxin-h5 (Trx-h5) during plant immune responses (Kneeshaw *et al.*, 2014). Interestingly, it has been suggested that Trx-h5 and GSNOR may denitrosylate a different set of protein-SNOs, which implies a high specificity for regulating the protein-SNO content in plants. However, how these denitrosylase enzymes select particular protein-SNO targets remains to be determined (Mata-Pérez and Spoel, 2019). Unravelling the functioning of *in vivo* denitrosylation would be a fruitful target of future research to definitively decipher the role of SNO during plant responses to stress. It has recently been shown that nucleoredoxin1 (NRX1) can protect cells from the oxidative stress generated under pathogen challenge by protecting antioxidant enzymes (Kneeshaw *et al.*, 2017). However, whether this type of thioredoxin is also involved in SNO homeostasis via the denitrosylation process remains to be determined. Whether NRX1 is involved during abiotic stress responses also requires further investigation. An SNO reductase that specifically breaks down S-nitrosated coenzyme A (SNO-CoA) and controls total SNO content has been described in animals. The deletion of this SNO-CoA reductase increases SNO levels and has an impact on the CoA metabolism (Anand *et al.*, 2012). The control of SNO content by SNO-CoA is a conserved mechanism from bacteria to mammals, but whether it also exhibits a conserved mode of action in plants needs further investigation.

Cellular functions of S-nitrosothiols

Despite of the importance of NO in the regulation of a wide range of processes in plants, there is still a gap in our knowledge about how this NO is produced in plants (reviewed by Astier *et al.*, 2018). One of the most intensive debates concerns the possible existence of a typical nitric oxide synthase (NOS) similar to that found in animals (Corpas and Barroso, 2017; Astier *et al.*, 2018). NOS-like activity has been identified in plants but a canonical NOS appears not to be present in higher plants (Jeandroz *et al.*, 2016; Corpas and Barroso, 2017). Interestingly, it has recently been proposed that different peptides work together to produce NO in a NOS-like form (Corpas and Barroso, 2017). As a consequence of the difficulties in identifying the specific sources of NO, researchers have focused their studies on downstream signaling events in order to decipher the importance of NO within plants. NO can interact with different macromolecules such as proteins, fatty acids, and nucleic acids, and particular attention has been given to protein modulation by NO via post-translational modifications (PTMs), such as the S-nitrosation modification that produces SNOs. In recent decades, extensive research looking for S-nitrosated targets has resulted in the identification of hundreds of protein-SNOs that are mainly involved in stress-related processes, metabolism, signaling, plant defense, and photosynthesis, among other physiological processes (Lindermayr *et al.*, 2005; Romero-Puertas *et al.*, 2007, 2008; Abat and Deswal, 2009; Kato *et al.*, 2013; Chaki *et al.*, 2015; Hu *et al.*, 2015). Some of the SNOs whose functional relevance has been well characterized at the molecular level are shown in Table 1.

Following identification of the proteins, the development of robust and powerful genomic tools such as microarrays and, in particular, RNA-seq has led to the identification under both physiological and stress-response conditions of thousands of NO-responsive genes that provide valuable insights into NO-signaling events (Parani *et al.*, 2004; Ferrarini *et al.*, 2008; Palmieri *et al.*, 2008; Ahlfors *et al.*, 2009; Begara-Morales *et al.*, 2014b). However, further studies are required to comprehensively characterize the impact of NO on these targets and their related signaling processes.

As noted above, SNOs are labile compounds so it can be a challenge to examine the effects of these redox molecules on plant responses to stress. In addition, it is not easy to differentiate

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Table 1. *Functional characterization of protein S-nitrosothiols in plants*

Species	Protein	Effect[1]	Target Cys[2]	Functional category	References
Arabidopsis thaliana	Transcription factor ABI5	–	153	Seed germination	Albertos *et al.* (2015)
	Snrk2.2	–	nd		Wang *et al.* (2015a)
	Snrk2.3	–	nd		
A. thaliana	Snrk2.6	–	137	Stomatal closure	Wang *et al.* (2015b)
A. thaliana	Transcription factor VND7	–	264, 320	Xylem differentiation	Kawabe *et al.* (2018)
A. thaliana	Rubisco large subunit	–	175	Photosynthesis	Fares *et al.* (2011)
Kalanchoe pinnata					Abat *et al.* (2008)
Brassica juncea					Abat and Deswal (2009)
A. thaliana	Metacaspase ATMC9	–	147	Protein cleavage	Belenghi *et al.* (2007)
A. thaliana	Glyceraldehyde phosphate dehydrogenase (GADPH)	–	155, 159	Metabolism	Holtgrefe *et al.* (2008);
Nicotiana tabaccum					Wawer *et al.* (2010)
A. thaliana	Methionine adenosyltransferase	–	114		Lindermayr *et al.* (2006)
A. thaliana	Transcription factor NPR1	–	156	Signaling-Plant immunity	Tada *et al.* (2008)
A. thaliana	Salicylic acid-binding protein 3 (AtSABP3)	–	280		Wang *et al.* (2009)
A. thaliana	Zinc finger SRG1	–	87		Cui *et al.* (2018)
A. thaliana	Transcription factorTGA1	+	172, 287, 260	Signaling	Lindermayr *et al.* (2010)
A. thaliana	Auxin receptor TIR1	+	140		Terrile *et al.* (2012)
A. thaliana	Transcription factor MYB2	–	53		Serpa *et al.* (2007)
Pisum sativum, *A. thaliana*	APX	+	32	ROS generation/scavenging	Begara-Morales *et al.*, (2014)
					Yang *et al.*, (2015)
P. sativum	MDAR	–	68		Begara-Morales *et al.* (2015)
P. sativum	GR	=	nd		Begara-Morales *et al.* (2015)
A. thaliana	DHAR	–	20, 147		Fares *et al.* (2011); Kato *et al.* (2013);
Solanum tuberosum					Puyaubert *et al.* (2014)
A. thaliana	PRXIIE	–	121		Romero-Puertas *et al.* (2007)
A. thaliana	NADPH oxidase	–	890		Yun *et al.* (2011)

[1] +, increased; –, decreased; =, not changed.
[2] nd, not determined.

SNOs from other reactive signaling molecules that are also generated under abiotic stress conditions, especially NO (Hancock and Neill, 2019). In this context, a general strategy used to determine the mode of action of SNOs has been the use of transgenic lines that have increased or decreased SNO levels (Feechan *et al.*, 2005). This has allowed the potential involvement of SNOs in response to abiotic stresses to be identified; however, more detailed characterization of their impact on the pathways in which they are involved is still required.

In the following section, we consider the involvement of protein-SNOs under different abiotic conditions, highlighting those signaling pathways in which a molecular characterization of the SNOs has been performed.

Signaling events mediated by S-nitrosothiol in plant responses to abiotic stress

Abiotic stresses are characterized by the over-production of ROS and RNS, which can ultimately compromise plant survival. Crop plants are often very sensitive to climate changes and consequently their final yields can be dramatically affected. Understanding how plants respond to environmental fluctuations by determining the signaling mechanisms involved in their tolerance to adverse conditions will be of a huge value for the development of crops that are more resilient to different types of abiotic stress. One of the signaling events during responses to environmental variations appears to be mediated by S-nitrosation. Indeed, it is well established that different abiotic stresses can induce SNO formation and the use of different pharmacological NO donors has been shown to regulate the activities of the main plant antioxidant enzymes (Procházková *et al.*, 2014; Arora *et al.*, 2016; Begara-Morales *et al.*, 2016b). In addition, the development of the biotin switch method, which allows the NO group of a SNO to be replaced by biotin (Jaffrey and Snyder, 2001), has allowed the identification of hundreds of protein-SNOs under normal physiological and stress conditions in many different species. Most of these S-nitrosation targets are redox-related proteins that are mainly involved in ROS generation and scavenging, which suggests an active role of SNOs in the control of redox homeostasis (Begara-Morales *et al.*, 2016a). Interestingly, the accumulation of SNOs in Arabidopsis *gsnor1* mutants leads to the up-regulation of antioxidant-related genes and an enhanced GSH-dependent antioxidant capacity compared to the wild-type, suggesting the participation of these enzymes in protection against the oxidative stress (Kovacs *et al.*, 2016) (Fig. 2). This implies that an excessive accumulation of SNOs could enhance the antioxidant capacity and contribute to tolerance to abiotic stresses that develop with an increase in the oxidative stress. It has been

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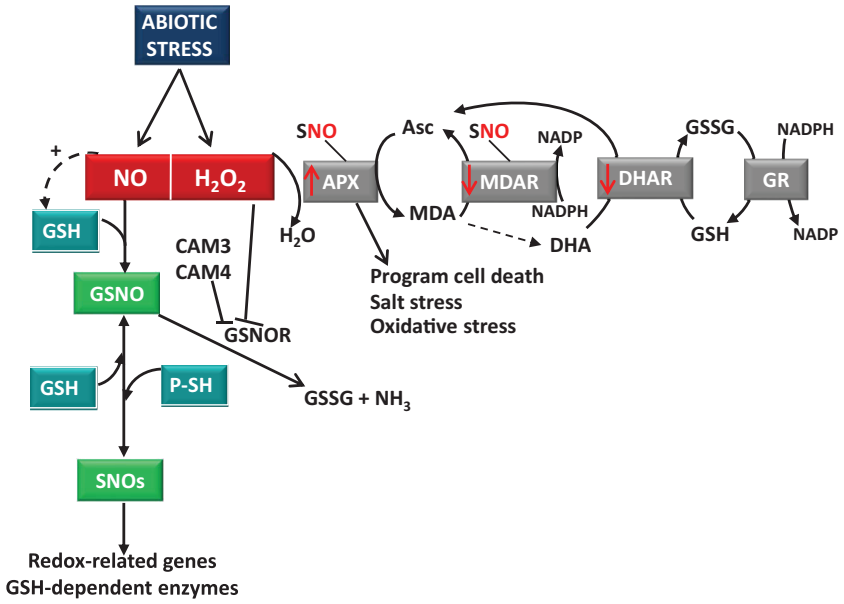

Fig. 2. Role of S-nitrosothiols in plant responses to abiotic stress. Different abiotic stresses trigger the production of NO and H_2O_2 that in turn regulate the signaling pathways leading to an adaptive response to the stress. The total accumulation of SNOs enhances the antioxidant response in deficient Arabidopsis *gsnor* mutants (Kovacs *et al.*, 2016). Salinity stress can induce the expression of calmodulin 3 (CAM3) and CAM 4 that inactivate S-nitrosoglutathione reductase (GSNOR) and therefore favor SNO accumulation (Zhou *et al.* (2016). The H_2O_2 generated during the nitro-oxidative stress can also inactivate GSNOR (Kovacs *et al.*, 2016) and therefore lead to SNO accumulation. This accumulation under abiotic stress can lead to the regulation of the ascorbate–glutathione (Asc–GSH) cycle by enhancing the function of ascorbate peroxidase (APX) and by negatively modulating monodehydroascorbate reductase (MDHAR) (Begara-Morales *et al.*, 2014a, 2015) and dehydroascorbate reductase (DHAR). DHA, dehydroascorbate; GR, glutathione reductase; GSSG, oxidized glutathione; MDA, monodehydroascorbate; PSH, thiol group of the protein;

proposed that the formation of SNOs could be a protective mechanisms against oxidative stress by avoiding the oxidation of critical Cys residues to an irreversible oxidized form (Jasid *et al.*, 2006; Abat and Deswal, 2009; Tanou *et al.*, 2009; Lounifi *et al.*, 2013; Begara-Morales *et al.*, 2014a; Tamura *et al.*, 2015). In addition, the main antioxidant systems are regulated by NO-PTMs (Begara-Morales *et al.*, 2016b), but their regulation by NO is sometimes contradictory (Fancy *et al.*, 2017). Furthermore, the accumulation of SNOs does not always confer resistance to an abiotic stress and sometimes the sensitivity is actually increased (Lee *et al.*, 2008; Gong *et al.*, 2015). These different effects could simply be the consequence of the diversity of the stresses that have been examined, the different strategies used to produce mutants with increased SNOs, and the differences in the severity and/or duration of the stresses imposed (Fancy *et al.*, 2017). SNOs could therefore have different effects in different species depending on the specific conditions imposed. Nevertheless, the mode of action of SNOs during responses to abiotic stress appears to be related to the control of redox homeostasis by modulation of the antioxidant capacity (Fig. 2). As noted above, SNO formation takes place in diverse organelles and consequently the model proposed in Fig. 2 could take place in different subcelullar localities.

Salinity

Salinity is one of the abiotic stresses that most affects crop yield and productivity worldwide (Saddhe *et al.*, 2018), and it is probably the environmental factor for which the most information about S-nitrosated proteins is available. The metabolism of SNOs has been shown to be affected during the response to salt stress in a number of different plant species, including olive (Valderrama *et al.*, 2007), pea (Camejo *et al.*, 2013; Begara-Morales *et al.*, 2014a, 2015), tomato (Kubienová *et al.*, 2014; Manai *et al.*, 2014), and citrus (Tanou *et al.*, 2009; Ziogas *et al.*, 2013). S-nitrosation together with other NO-PTMs mediated by different RNS are an important part of the signaling mechanisms that lead to plant responses to salt stress (reviewed by Saddhe *et al.*, 2018). It has been shown that GSNOR is involved in tolerance to salt stress in Arabidopsis by acting downstream of calmodulin 1 (CAM1) and CAM4. Zhou *et al.* (2016) found that salt stress induced the expression of CAM1 and CAM4 together with an increase in NO levels. Interestingly, the deficient mutants *cam1* and *cam4* accumulated less NO and exhibited a higher sensitivity to salt stress than wild-type plants. This decrease in NO levels appeared to be related to inhibition of GSNOR activity by calmodulin during salt stress (Zhou *et al.*, 2016). Furthermore, *gsnor* transgenic plants that accumulated NO/SNOs exhibited a typical dwarf phenotype but were more tolerant to salt stress than the wild-type; the tolerance or sensitivity of the mutants was restored using complementation transgenic lines. Zhou *et al.* (2016) concluded that the inactivation of GSNOR by CAM1 and CAM4 allowed the accumulation of NO/SNO, which in turn conferred resistance to salt stress in the plants. The regulation of SNO content by an S-nitrosation/denitrosation mechanism appears to determine the response to stress in sunflower seedlings. Jain *et al.* (2018) found that 120 mM NaCl inhibited GSNOR activity and resulted in the accumulation of SNOs in sunflower cotyledons and roots; however, this had a different effect depending on the tissue, with S-nitrosation increasing in the cotyledons and denitrosylation increasing in the roots.

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Following biotin switch assays coupled to mass spectrometry, a set of S-nitrosated target proteins were identified in both organs, some of which were related to redox homeostasis, namely monodehydroascorbate reductase (MDAR), ascorbate peroxidase (APX), and peroxiredoxin. Interestingly, it was shown that MDAR activity was differentially regulated after salt treatment depending on the organ. Salt stress induced S-nitrosation of MDAR and thus inhibited its enzymatic activity in the cotyledons, whereas denitrosation of the enzyme enhanced its action in the roots (Jain *et al.*, 2018). It is important to note that independent of the different response to salt stress, S-nitrosation inhibited MDAR activity in both organs. Similarly, MDAR function has also been proposed to be negatively regulated by S-nitrosation in pea leaves subjected to salt stress. Begara-Morales *et al.* (2015) found that S-nitrosation of Cys68 appeared to be responsible for this inhibition. Interestingly, glutathione reductase (GR), another component of the ascorbate–glutathione cycle (Asa–GSH), was not affected in its function after S-nitrosation. APX has emerged as a crucial point in the interplay between ROS and RNS signaling (Lindermayr and Durner, 2015). Cytosolic APX exhibits dual regulation by NO-PTMs under salt stress in pea leaves: tyrosine nitration negatively affects APX activity whereas S-nitrosation of Cys32 is proposed to enhance its function and therefore contribute to the adaptation to salt stress (Begara-Morales *et al.*, 2014a) (Fig. 2). In addition, auxin-mediated denitrosation inhibits APX activity, which has an impact on root architecture (Correa-Aragunde *et al.*, 2013, 2015). Another piece of evidence for this regulation of APX is the fact that genomic analysis has shown that S-nitrosation of Cys32 is responsible for increasing its activity, and that this residue is important during the response to oxidative stress and in plant immunity (Yang *et al.*, 2015).

The promotion of seed germination and plant growth by NO in Arabidopsis under salt stress have been proposed to depend on ETHYLENE INSENSITIVE 3 (EIN3). Li *et al.* (2015) showed that NO favors EIN3 accumulation and therefore it can control EIN3-downstream signaling events during the adaptive response to salt stress. However, the improved germination under salt stress by NO could be a consequence of the crosstalk between NO and abscisic acid (ABA) in the regulation of seed dormancy. NO promotes seed germination (León *et al.*, 2014) via regulation of ABA-related dormancy at multiple levels (Signorelli and Considine, 2018). For instance, NO accumulation triggers the S-nitrosation of ABA INSENSITIVE 5 (ABI5), a master regulator of ABA-dependent seed dormancy and early plant growth (Skubacz *et al.*, 2016), at Cys153 (Albertos *et al.*, 2015). This modification favors ABI5 degradation by proteosomes and therefore promotes seed germination (Albertos *et al.*, 2015). Furthermore, the S-nitrosation of the SnRK 2.2 and SnRK 2.3 proteins negatively regulates ABA-mediated seed germination and early plant growth (Wang *et al.*, 2015a). However, whether these proteins are regulated by S-nitrosation during salt stress requires further studies to determine the crosstalk between ABA and NO during the control of seed germination under these conditions.

It has recently been proposed that S-nitrosation is able to regulate other post-translational modifications such as protein methylation during plant responses to stress in Arabidopsis (Hu *et al.*, 2017). Different genomic approaches using PROTEIN METHYLTRANSFERASE 5 (PRTM5) and GSNOR1 deficient and overexpressing mutants showed that S-nitrosation of PRTM5 at Cys125 positively regulated its methyltransferase activity and consequently conferred salt tolerance (Hu *et al.*, 2017). This response appeared to be related to the modulation of splicing of stress-related pre-mRNA after PRMT5 S-nitrosation.

Other studies have identified some specific protein targets of S-nitrosation during plant responses to salt stress. Some of these proteins are related to redox metabolism, such as APX, Fe-SOD, MDAR, and glutaredoxin (Tanou *et al.*, 2009, 2012; Fares *et al.*, 2011; Camejo *et al.*, 2013). Exogenous application of the NO donor S-nitroso-N-acetylpenicillamine (SNAP) decreases the negative effects of salinity stress by enhancing the antioxidant systems in chickpea (Ahmad *et al.*, 2016). However, there has been no in-depth characterization of the functions of these SNOs in plant adaptation to salt stress. More studies using mutant lines with increased and decreased SNOs levels would be of great help in comprehensively deciphering the real impact of these redox molecules on the signaling mechanisms that lead to plant adaptation to salt stress. This information would help in developing future crops that are more tolerant to salt stress.

Extreme temperatures

High temperature is one of the main environmental stresses that limits plant growth since it negatively affects vegetative and reproductive growth (Li *et al.*, 2018), which has important implications for agriculture. After perceiving high temperature, plants trigger a signaling response in which heat-shock proteins, hormones, ROS, and calcium act together to produce an adaptive response (Parankusam *et al.*, 2017; Li *et al.*, 2018). Interestingly, the endogenous occurrence of nitrolinolenic acid (NO_2-Ln) has recently been reported for the first time in plants (Mata-Pérez *et al.*, 2016b). Using RNA-seq analysis, it was shown that NO_2-Ln is mostly able to modulate the expression of genes related to abiotic stresses in Arabidopsis, with the major response being related to the induction of heat shock proteins and the chaperone network. Interestingly, treatment of human endothelial cells with another nitro fatty acid (NO_2-FA), nitro-oleic acid, also triggers a response mediated by heat-shock proteins (Kansanen *et al.*, 2009), therefore suggesting a conserved mechanism of action for NO_2-FAs. Moreover, the content of NO_2-Ln is modulated during Arabidopsis development and during responses to different abiotic stresses such as wounding, cadmium, or salt, implying that it could be implicated in these processes. However, how NO_2-Ln specifically regulates these processes needs further investigation: the identification by mass spectrometry of its protein targets and how they are functionally modulated would be a good starting point. It is also important to note that NO_2-Ln is able to release NO (Mata-Pérez *et al*, 2016a) and therefore it could be implicated in NO-related signaling events during plant responses to abiotic stresses.

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One of the first genetic analyses to highlight the importance of NO in plant thermotolerance was performed using HOT5/GSNOR-deficient mutants that accumulated more nitrate and nitroso species, which led to an increased sensitivity to heat stress (Lee *et al.*, 2008). However, there is no consensus about the role of NO/SNOs during the response to heat stress. For example, transgenic Arabidopsis lines with deficient NO production exhibit a lower survival rate after heat treatment compared to wild-type plants (Xuan *et al.*, 2010), and it was suggested that NO acts as a signal that acts upstream of CAM3 during the thermotolerance response. However, heat stress induces NO generation that promotes proline accumulation and antioxidant systems in *Vicia faba*, leading to thermotolerance (Alamri *et al.*, 2018). In addition, in sunflower hypocotyls subjected to heat stress there is an increase in SNO content that is independent of NO production (Chaki *et al.*, 2011b). This accumulation of SNOs enhances tyrosine nitration, which in turn is responsible for the inhibition of proteins involved in photosynthetic carbon assimilation (Chaki *et al.*, 2011b). This confirms that NO/SNOs can act as fundamental regulators of the photosynthesis process (Misra *et al.*, 2017; Parankusam *et al.*, 2017). However, further studies are needed to identify the specific targets of SNOs and their involvement in the signaling events that are triggered by heat stress.

Low temperature is also a fundamental abiotic stress that affects plant survival by affecting photosynthesis and water uptake. During the course of this stress there is also a high response of RNS (Corpas *et al.*, 2008; Airaki *et al.*, 2012; Sehrawat *et al.*, 2013). Although there is not much information available regarding SNO signaling during plant responses to low-temperature stress, some specific functions have been proposed. Some pathogenesis, photosynthetic, and signaling proteins have been identified as being S-nitrosated in response to cold stress in *Brassica juncea*, with Rubisco being identified as the major protein to be modulated (Abat and Deswal, 2009). Thus, Sehrawat *et al.* (2013) used a depleted Rubisco fraction to identify protein-SNOs in response to cold stress, resulting in the identification of 15 targets. For example, Fe-SOD was identified to be positively regulated by S-nitrosation and therefore to contribute to the detoxification of superoxide radicals. In addition, 20 protein-SNOs have been identified during the response of Arabidopsis to cold stress (Puyaubert *et al.*, 2014). Molecular characterization of the impact of S-nitrosation on these target proteins would be a good starting point for improving our knowledge of the signaling mechanisms that produce plant responses to cold stress.

Overall, further studies are needed to decipher the specific roles of SNOs in plant responses to extreme temperatures. The identification of additional S-nitrosation targets and the functional relevance of SNOs in signaling events will be required to gain a better knowledge of the general response to this stress.

Drought stress

Drought stress induces severe effects on plant growth and crop productivity as a consequence of alterations in a multitude of physiological processes (Lisar *et al.*, 2012). One drought-related response is the accumulation of the phytohormone ABA (Lisar *et al.*, 2012), which is a central regulator of plant responses to abiotic stresses (Sah *et al.*, 2016). In addition, other signaling molecules are generated during drought/water deficit such as ROS and RNS, which can mediate nitrosative stress (Signorelli *et al.*, 2013). It is well established that crosstalk between NO and ABA signaling governs the regulation of a wide range of physiological and stress responses in plants (reviewed by Prakash *et al.*, 2019). Of special interest is the relationship between these signaling molecules during plant responses to drought stress, where they modulate stomatal opening, photosynthesis, proline accumulation, and seed germination (Santisree *et al.*, 2015). The exogenous application of a NO donor leads to water-deficit tolerance in *Crambe abyssinica* (Batista *et al.*, 2018). Interestingly, overexpression of rat neuronal NOS potentiates the tolerance of rice plants to drought stress as a consequence of alleviation of ROS-related damage by the enhancement of antioxidant functioning and drought-related gene expression (Cai *et al.*, 2015). Constitutive production of NO induces a transcriptional reprogramming in Arabidopsis, with two ABA receptors, AtPYL4 and AtPYL5, being important during drought resistance in mutant plants (Shi *et al.*, 2014). Conversely, the overexpression of the non-symbiotic haemoglobin gene *HvHb1*, which results in lower NO levels than in the wild-type, increases tolerance to drought stress in barley (Montilla-Bascón *et al.*, 2017). Interestingly, in transgenic plants with reduced endogenous levels of NO, an interplay between ABA and NO has been established in the context of seed germination and responses to water deficit (Lozano-Juste and León, 2010). The Arabidopsis triple-mutant *nia1 nia2 noa1* exhibits deficient biosynthesis of NO as a consequence of impairment of nitrate reductase (NR) and NO-ASSOCIATED 1 (NOA1). This mutant shows a lower germination rate than the wild-type and is hypersensitive to ABA, which suggests the control of ABA-induced dormancy by NO (Lozano-Juste and León 2010). The mutant is also more resistant to water deficit than the wild-type as the result of more efficient stomatal closure. This is not surprising since it is well known that NO regulates ABA-mediated stomatal closure (reviewed in Prakash *et al.*, 2019). This regulation appears to be mediated in Arabidopsis by the S-nitrosation of the Cys137 of the SnRK2.6 protein, a residue adjacent to the kinase catalytic site (Wang *et al.*, 2015b). Interestingly, accumulation of SNO in GSNOR-deficient mutants promotes an over-accumulation of S-nitrosated SNRK2.6, and hence an impairment in ABA-induced stomatal closure and an insensitivity to ABA.

Although this mechanism of regulation of drought stress by crosstalk between NO and ABA is well established, more information regarding S-nitrosated targets and their physiological relevance is required in order to comprehensively understand the NO-mediated signaling events that lead to the plant responses to drought (Santisree *et al.*, 2015).

It has recently been shown that flooding stress also induces changes in SNO metabolism (Hashiguchi and Komatsu, 2018), and the functional categories where these protein-SNOs are predicted to be involved are development, stress, and glycolysis. However, the specific role of the SNOs in these processes remains to be elucidated.

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Other stresses

Although alterations of SNOs and other RNS have been reported in various species subjected to several abiotic stresses, the specific role of these SNOs with regards to the signaling mechanisms that lead to plant survival has not been addressed.

Plants are exposed to various environmental stresses that can cause them mechanical injury, such as hail, rain, wind, and herbivores (Corpas *et al.*, 2011). Wounding stress usually causes loss of nutrients and also facilitates infection by pathogens (Savatin *et al.*, 2014). To protect themselves against mechanical injury, plants have a complex defense mechanism that involves physical barriers as well as hormones and a wide range of metabolites (Savatin *et al.*, 2014), and NO has been proposed to be part of the signaling events that mediate the response to wounding stress (Corpas *et al.*, 2008; Arasimowicz *et al.*, 2009; Chaki *et al.*, 2011b; Espunya *et al.*, 2012). For example, in sunflower 4 h after wounding stress inactivation of GSNOR led to accumulation of GSNO and SNOs, which mediated a nitrosative stress (Chaki *et al.*, 2011a). In addition, GSNO/SNOs have been proposed to act as a transporter of the wounding signal from the injured tissues to the rest of the plant through the vascular tissue (Espunya *et al.*, 2012; Houmani *et al.*, 2018). S-nitrosated proteins are reduced in extrafascicular phloem samples after wounding stress in pumpkin (Gaupels *et al.*, 2016). However, despite this evidence of the involvement of SNOs, information regarding their specific role within the signaling mechanisms that are triggered in response to wounding remain scarce.

Cadmium stress has been reported to modulate RNS metabolism (Barroso *et al.*, 2006; Rodriguez-Serrano *et al.*, 2009). It has been shown that S-nitrosation negatively regulates catalase and glycolate oxidase in pea peroxisomes and that this is reduced after cadmium stress (Ortega-Galisteo *et al.*, 2012). A total of 32 differentially S-nitrosated proteins have been detected in poplar leaves after ozone stress (Vanzo *et al.*, 2014), among which is the phenylalanine ammonia-lyase protein that is enhanced after de-nitrosylation derived from ozone stress (Vanzo *et al.*, 2014). An excessive accumulation of SNOs induces sensitivity to sodic alkaline stress in tomato plants (Gong *et al.*, 2015).

Conclusions and future perspectives

S-nitrosation has emerged as the main mechanism to transmit NO bioactivity during abiotic stress responses. -Responses to salinity and drought stress mediated by S-nitrosation are becoming well established via the regulation of antioxidant systems and ABA-mediated signaling, respectively. However, a more comprehensive characterization of S-nitrosation targets is still required to determine the physiological relevance of protein-SNOs in the signaling events that lead to plant tolerance to other abiotic stresses.

It is not an easy task to differentiate the SNO signal from other redox molecules generated during plant responses to abiotic stress, such as ROS, glutathione, other antioxidants, and especially NO (Hancock and Neill, 2019); however, the control of antioxidant systems by S-nitrosation has emerged as an interesting regulation point during abiotic stress. Major efforts are needed in order to clarify the specific control of ROS-related enzymes by S-nitrosation during the stress responses. In addition, we also need to determine how the functions of NO and SNO depend on the redox state in the cells under environmental stress, and how these signal molecules are connected to other redox-related molecules (Hancock and Neill, 2019).

Acknowledgements

JCBM wishes to thank the Ministry of Economy and Competitiveness (Spain) for postdoctoral research funding within the Juan de la Cierva-Incorporación program (IJCI-2015–23438). The work in our lab is supported by ERDF grants co-financed by the Ministry of Economy and Competitiveness (project PGC2018-096405-B-I00) and the Junta de Andalucía (group BIO286) in Spain.

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